Description of antennal structures of the parasitoid Mallophora ruficauda *(Diptera: Asilidae) and its relationship with resources searching behaviour*

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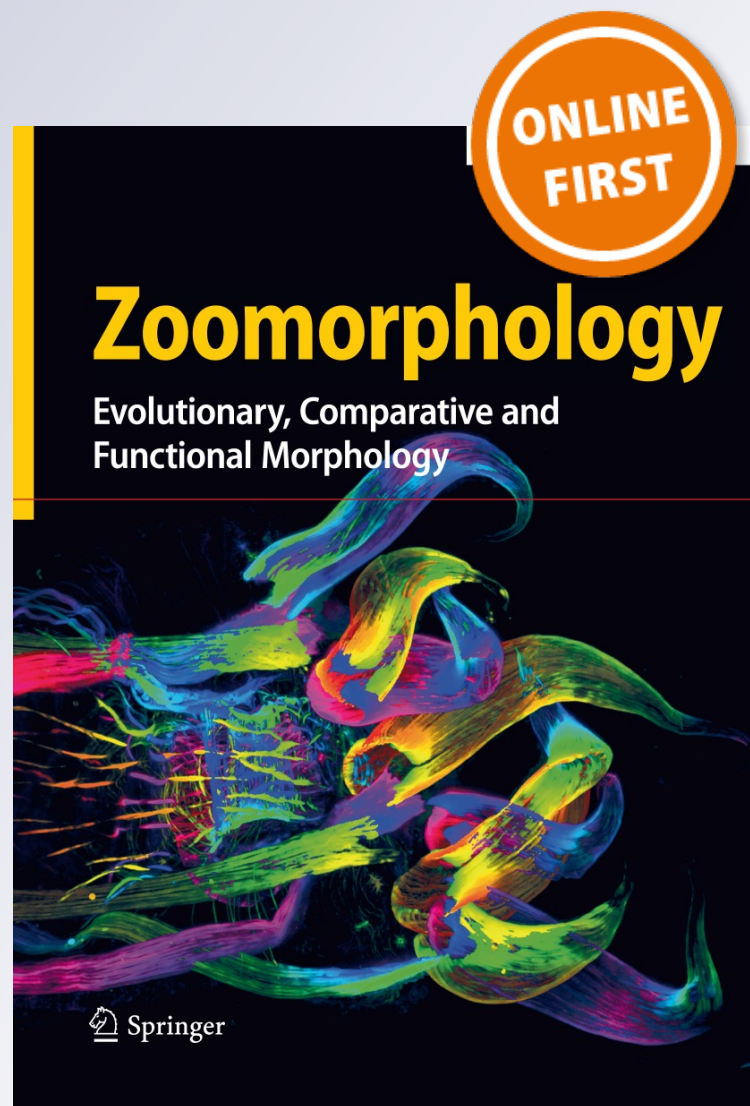
H. F. Groba · L. S. Lauria de Cidre ·
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Abstract The robber fly *Mallophora ruficauda* is a parasitoid of white grubs (Coleoptera: Scarabaeidae) inhabiting in Pampas region of Argentina. Females locate host's habitat and lay eggs away from the host in tall grasses. After hatching, larvae fall to the ground and actively seek hosts. Previous works suggested that female would detect the presence of host's chemical cues, but sensory organs involved in olfaction are still unknown. However, few studies have looked at dipteran parasitoids sensilla, and no study has been undertaken in Asilidae species. The aim of this work was to determine the presence, density, distribution and morphology of chemosensilla in *M. ruficauda* antennae using optic and scanning microscope techniques. We found that antennae have 4 segments: scape, pedicel, postpedicel and style. We identified basiconic and trichoid sensilla, small and long bristles, and sensory pits. Basiconic sensilla are multiporous and are widely spread between the small bristles through the postpedicel. Trichoid sensilla are grouped in 6–8 units on latero-ventral margin of postpedicel, have mobile base, striated wall and an apical porous. Small bristles are present in the pedicel and postpedicel, and long bristles are found in groups on scape and pedicel. Three different types of sensory pits were observed, with basiconic sensilla, distributed along external and internal lateral side of the postpedicel. Considering the morphological characteristics of the antennae, and based on the olfaction biomechanics, the structure and distribution of these cuticular structures of the parasitoid antennae would contribute to the odour detection mechanism in adults of *M. ruficauda*.

Keywords Sensilla · Olfaction · Chemoreception · Parasitoid · Asilidae

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Introduction

Location of resources involves many cues or stimuli from the environment that are detected by individual's organ senses. For insects, the functional and structural sensorial unit that detects chemical, mechanical and thermo-hygro stimuli is denominated sensillum (Zacharuk 1980; Kaissling 1986; Snodgrass 1997; Ryan 2002; Chapman 2013). Antennae of adult insects are important sensory structures that bear various types of sensilla with different functions. They play an important role in several behaviours during adult life, such as habitat odour recognition, mate searching, predator avoidance and resource location (Quicke 1997; Snodgrass 1997; Ryan 2002; Sato and Touhara 2009; Chapman 2013). Particularly for parasitoids, antennal sensilla are important during searching and locating hosts or their microhabitat, during host discrimination and

acceptance, and oviposition site selection (Vet et al. 1995; Roux et al. 2005; Da Rocha et al. 2007; Gao et al. 2007; Onagbola and Fadamiro 2008; Dweck 2009; Romani et al. 2010; Wang et al. 2010; Das et al. 2011; Obonyo et al. 2011; Xi et al. 2011; Zhou et al. 2011, 2013). Commonly, parasitoids use olfactory or/and gustatory sensilla during a given searching behaviour, depending on the host habitat they exploit (Vet and Dicke 1992; Turlings et al. 1995, Roux et al. 2005; Da Rocha et al. 2007; Gao et al. 2007; Onagbola and Fadamiro 2008; Dweck 2009; Das et al. 2011; Obonyo et al. 2011). Therefore, studies of antennal morphology, types of sensilla and their structure are important to understand behaviour variations in parasitoid species (Turlings et al. 1995; Roux et al. 2005; Da Rocha et al. 2007; Gao et al. 2007; Van Baaren et al. 2007; Onagbola and Fadamiro 2008; Das et al. 2011; Xi et al. 2011).

Hymenopteran parasitoids searching for hosts use different kind of stimuli that allow them to find hosts efficiently. Once the encounter takes place, females lay eggs into or onto hosts (Van Alphen and Vet 1986; Godfray 1994; Quicke 1997). On the contrary, many dipteran parasitoids have a split host seeking behaviour where females locate the host habitat, lay eggs away from the host, and then, larvae search actively and find the host (Godfray 1994; Feener Jr. and Brown 1997; Fournet et al. 2001; Brodeur and Boivin 2004; Ho et al. 2011; Ichiki et al. 2012; Goubert et al. 2013). Female parasitoids with this host searching strategy would increase the probability of encounter between the larva and the host, enhancing the location of the host habitat, through a developed olfaction system that would allow them to detect hosts at different habitat spatial scales. However, relatively little information exists on the antennal sensilla of dipteran parasitoids (Giangiuliani et al. 1994; Rahal et al. 1996; Abouzied 2008; Chen and Fadamiro 2008), and virtually none for parasitoids with split searching behaviour, as Asilidae family.

The members of the Asilidae family are dipterans commonly known as robber flies with larvae that attack larvae of scarab beetles (Hull 1962; Clements and Bennett 1969; Wood 1981; Musso 1983; Castelo and Lazzari 2004). Larvae search and attack their host underground, which are killed and consumed immediately or are kept alive and killed several months later (Hull 1962; Clements and Bennett 1969; Musso 1983; Wood 1981). Adult asilid flies are predators of other flying insects, which are hunted using visual cues (Wood 1981; Musso 1983; Shelly 1984; O'Neill 1992a, b). A few species, all belonging to the *Mallophora* genus, are regarded as parasitoids, because during their larval stages they attack white grubs and consumed them slowly (Clements and Bennett 1969; Wood 1981; Musso 1983; Castelo and Lazzari 2004). *Mallophora ruficauda* (Wiedemann) 1828 is endemic of

the Pampas region of Argentina inhabiting open grasslands near bee farms. As adults, *M. ruficauda* feeds mainly on foraging honeybees and other flying insects, and as larvae, they are parasitoids of white grubs (Coleoptera: Scarabaeidae), mainly third instar larvae of *Cyclocephala signaticollis* Burmeister (Castelo and Lazzari 2004; Castelo and Corley 2010). During their adult lifespan (December–March), mated females deposit eggs in clusters on elevated available sites, as natural tall vegetation or artificial supports (Castelo and Corley 2004). Emerging larvae drop into the soil, dig and search for hosts, using chemical cues (Castelo and Lazzari 2004; Crespo and Castelo 2008; Groba and Castelo 2012). There are ecological studies that suggest that oviposition sites could be linked to cues related to host presence in the environment, proposing that oviposition behaviour is induced by chemical cues arisen from *C. signaticollis* larvae feeding on *Carduus acanthoides* (Castelo and Corley 2004), fact that has been suggested for other robber flies species (Kershaw 1912; Hardy 1929). However, previous works suggested that there would be no plant cues associated with *M. ruficauda* oviposition behaviour because females lay their egg clusters also on dead plants and artificial supports such as wire fences, and also showed that the oviposition site height is involved in this behaviour (Castelo and Corley 2004; Castelo et al. 2006). Nevertheless, *M. ruficauda* females could use different types of cues in their seeking behaviour like other parasitoids do (Godfray 1994; Vet et al. 1995; Feener and Brown 1997; Stireman III 2002; Stireman III et al. 2006; Ichiki et al. 2012; Goubert et al. 2013). At a big scale, chemical cues are important to locate hosts, and at small scale, oviposition site height maximizes the encounter with the host (Castelo et al. 2006). However, no studies have looked at the sensilla of *M. ruficauda* adults providing descriptions of the antennal chemosensory receptors involved in detection of chemical cues, hence, supporting this hypothesis.

The typical insect antenna is composed by three main parts: a proximal scape, a pedicel and a distal flagellum, the latter often divided into several number of parts or flagellomeres (Snodgrass 1997; Sato and Touhara 2009; Hu et al. 2010; Romani et al. 2010; Hansson and Stensmyr 2011; Wang et al. 2012; Chapman 2013; Zhang et al. 2013a, b). It is common that both the scape and pedicel bear mechanosensory sensilla/organs, while it is in the flagellum where most of the olfactory sensilla are located (McAlpine 1981; Hu et al. 2010; Hansson and Stensmyr 2011; Wang et al. 2012; Chapman 2013; Zhang et al. 2013a, b). In higher Diptera (i.e. Brachycera) as species of Asilidae, the first flagellomere (postpedicel or funiculus) is endowed with sensory pits and almost all sensilla are important in the detection of chemical cues (Hallberg et al.

1984; Stocker 1994; Rahal et al. 1996; De Freitas Fernandes et al. 2002, 2004; Wasserman and Itagaki 2003; Sukontason et al. 2004, 2007, 2008; Chen and Fadamiro 2008; Smallengange et al. 2008; Castrejon-Gomez and Rojas 2009; Hu et al. 2010; Poddighe et al. 2010; Bisotto-de-Oliveira et al. 2011; Setzu et al. 2011; Wang et al. 2012; Zhang et al. 2013a, b). In Asilidae species, the antenna is composed of four segments, the scape and pedicel often with stout bristles, and two flagellomeres, the pubescent but without bristles postpedicel, and the stylus that is usually bi-segmented, occasionally singly segmented (Wood 1981; Yeates 2002). Although there are some descriptions, there is no available information about morphology, composition and structure of antennal sensilla in this group.

In the present study, we described the external antennal morphology of female and male *M. ruficauda*, using optic (OM) and scanning electron microscopy (SEM) techniques with emphasis in the olfactory sensilla in the postpedicel, and compared the type, length and density of other cuticular structures between sexes. This knowledge will provide a morphological basis to understand the process of host location in the adult of this parasitoid.

Fig. 1 General morphology of *M. ruficauda* antennae. **a** Natural position of antennae in the adult. **b** SEM micrograph of the antenna. I = scape, II = pedicel, III = postpedicel, IV = terminal stylus. *Scale bar* = 500 μm

Materials and methods

Insects

We collected adults of *M. ruficauda* by hand and directly from the plants using a flask in a grassland of a bee farm in Moreno (34°46′S, 58°93′W), Buenos Aires province, Argentina, from January to February of 2008. Once in the laboratory, we anesthetized the individuals with CO_2 and then fixed them with 80 % alcohol.

Morphology of the antennae

In order to characterize the general antennal morphology, we made observations with an OM. We dissected antennae from six females and six males and then conventionally hydrated them in graded alcohol series, and placed in hydrogen peroxide (30 % v/v) for 48 h. Subsequently, we dehydrated the antennae conventionally up to 100 % alcohol. Then, we placed the pieces for 3 min in the stove, clearing them with xylol (15–30 min) and mounted them with the common mounting medium DPX (distyrene–plasticizer–xylene).

We made general measurements of external parts of the antenna using digital photograph. Once images were obtained, maximum width, length and total area of each segment of the antenna (left or right) and maximum length
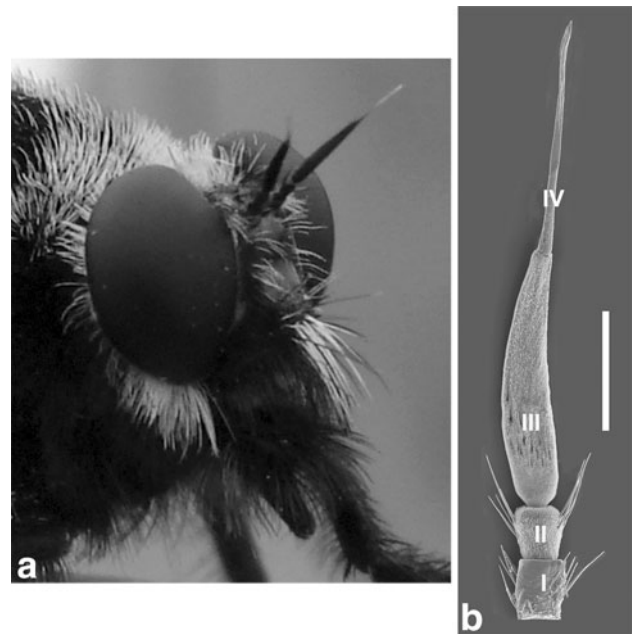
and width of the sensory pits were registered. Measurements were taken using an image measurement software (Golden Ratio 1.1, http://www.markuswelz.de; ImageJ, http://rsbweb.nih.gov/ij/; Adobe Photoshop CS2 v 9.0.2).

Description of cuticular structures

To analyse the composition, size and the three-dimensional array of the sensilla and other cuticular structures, we prepared the antennae for SEM, employing a protocol for daphnids species modified from Laforsch and Tollrian (2000). Briefly, seven female and seven male heads were dissected, hydrated up to tap water and then placed in a non-ionic detergent solution (0.3 % v/v) for 30 min. The heads were then rinsed with the aid of ultrasound (∼20 kHz) for 2.5 min to loosen up undesirables particles. Heads, after rinsed, were dehydrated in a graded series of distilled water, 50 and 70 % alcohol during 30 min each. Antennae were dissected, and subsequently dehydration continued with 80 %, 90 %, 2 × 96 % and 2 × 100 % alcohol for 25 min each. To completely dry the samples and to avoid sensilla deformation, antennae pairs were immersed in 4 ml of hexamethyldisilazane (1,1,1,3,3,3-hexamethyldisilazane; Sigma-Aldrich, Argentina) in 12 ml glass vials. After a soak of 50 min, approximately 90 % of the hexamethyldisilazane was removed and the vials were immediately transferred to a desiccator with silica gel. The

Table 1 Measurements of the antennal segments of *M. ruficauda*

	Measure	♀ [N = 6]	♂ [N = 6]	t; p
Antennae	Total length	2.49–3.10 (2.88 ± 0.22)	2.17–3.04 (2.70 ± 0.34)	1.11; 0.293
	Maximum width	0.27–0.30 (0.29 ± 0.01)	0.21–0.31 (0.26 ± 0.04)	1.82; 0.139
Scape (I)	Maximum length	0.33–0.42 (0.38 ± 0.01)	0.26 ± 0.40 (0.33 ± 0.05)	1.87; 0.090
	Maximum width	0.27–0.30 (0.29 ± 0.01)	0.21–0.31 (0.26 ± 0.04)	1.82; 0.139
Pedicel (II)	Maximum length	0.25–0.29 (0.27 ± 0.02)	0.20–0.28 (0.26 ± 0.03)	1.17; 0.270
	Maximum width	0.22–0.25 (0.24 ± 0.01)	0.16–0.24 (0.20 ± 0.02)	2.98; 0.014*
Postpedicel (III)	Maximum length	1.02–1.27 (1.17 ± 0.09)	0.88–1.33 (1.14 ± 0.18)	0.42; 0.686
	Maximum width	0.26–0.28 (0.26 ± 0.01)	0.20–0.26 (0.23 ± 0.02)	3.44; 0.006*
	Area	0.43–0.54 (0.46 ± 0.04)	0.33–0.57 (0.44 ± 0.09)	0.30; 0.768
Terminal stylus (IV)	Maximum length	0.98–1.18 (1.07 ± 0.07)	0.82–1.04 (0.96 ± 0.08)	2.43; 0.036*
	Maximum width	0.046–0.059 (0.052 ± 0.005)	0.038–0.059 (0.048 ± 0.007)	1.30; 0.223

The ranges of measurements are followed between brackets by the mean and SD. *Asterisks* denote statistically significant differences (*t*, $p < 0.05$). All measurements are in mm, except for the postpedicel area that is in mm^2. Between square brackets, the total number of measured individuals (*N*) is expressed

remaining hexamethyldisilazane was allowed to evaporate overnight under anhydrous conditions. Following the hexamethyldisilazane drying, antennae were mounted on aluminium stubs with carbon tape and sputter coated with gold. Antennae were examined and photographed with a Carl Zeiss Supra 40 SEM. The terminology and classification of sensilla types were done on the basis of cuticular morphology and ultrastructure, and were compared with sensilla already described in bibliography (Zacharuk 1980; Wood 1981; Snodgrass 1997; Ryan 2002; Chapman 2013). We then characterized type, length and density of the different structures found.

For sensilla and cuticular structures type identification, and assessment of their length and density, digital photographs of the SEM technique of an antenna portion of 0.007 mm^2 were used. As funiculus or postpedicel bears the majority of olfactory sensilla in insects, we made the measurements only in the postpedicel of the *M. ruficauda* antennae. We defined and photographed three zones along the postpedicel, which are proximal, medial and distal. Sensillum mean length was estimated from the base to the tip of the sensillum (N = 15) in each postpedicel zone per individual (females N = 7, males N = 7). Density of sensilla was estimated in one antenna of each individual using a subsample of a 400 μm^2 square. In total, 12 measurements were done at random per individual along the photograph for each postpedicel zone (females N = 7, males N = 7). However, the measurements were made either on the right or the left antenna of the individual depending on the position or condition of the structure. Then, for each measurement, the mean number of sensilla inside the square was registered. In order to avoid pseudoreplication during the measurements, we guarantee that there was no superposition in the squares between the measurements. We also performed this analysis to assess the occurrence of variations in the olfactory sensilla, as in size and composition of sensilla and other cuticular structures among postpedicel zones. Through the OM photographs, we obtained the number and length of the long bristles present in the scape and pedicel. We then made the average between measures from the left and right antenna of each individual analysed.

In order to estimate the number of basiconic sensilla in the postpedicel in both sexes, we calculated the product between the average density of the sensilla present in the medial and distal zone from the images obtained from SEM. Also, we used the area of the antennal segments of the images obtained by the OM. Details of estimations of density and area were explained in the previous paragraph.

Comparison of antennal morphology

In order to determine whether there exists heterogeneity in morphology of *M. ruficauda* antennae, we analysed the total width and length of the antenna and of each segment, the total area of the postpedicel, and, the number, maximum width and length of the postpedicel sensory pits measuring the structures on OM images. Then, we calculated the average of each measurement and evaluated the difference between the two sexes by means of a two-tailed *t* test (Zar 2010). To analyse whether the size and three-dimensional array of sensilla differ between sexes, we compared the length and density of sensilla by a repeated measures analysis of variance (ANOVA) for each type of sensilla in postpedicel zones (Quinn and Keough 2002). We transformed the variables postpedicel total area;

Fig. 2 Digital photograph of *M. ruficauda* antennae. **a** SEM micrograph showing postpedicel (*scale bar* = 150 μm). **b** Digital photograph of OM preparation of external side of postpedicel showing sensory pits (ov, oval; cl, cloud) (*scale bar* = 70 μm). **c** Digital photograph of OM preparation of internal side of postpedicel showing sensory pits (sc, sack) (*scale bar* = 70 μm). **d** Detail of a sensory pit (*scale bar* = 8 μm). **e** Disposition of trichoid sensilla in the proximal zone grouped in the latero-ventral region of the postpedicel (*scale bar* = 100 μm)

number, maximum length and width of postpedicel sensory pits; and density of small bristles in order to meet assumptions of the analysis. Statistical analyses were done using the software package Infostat (version 10.1, FCA, Universidad Nacional de Córdoba, Argentina) and SPSS for Windows, Release version 17.0 (© SPSS, Inc., 2008, Chicago, IL, www.spss.com). A $p < 0.05$ was considered to state that differences between statistics were statistically significant.
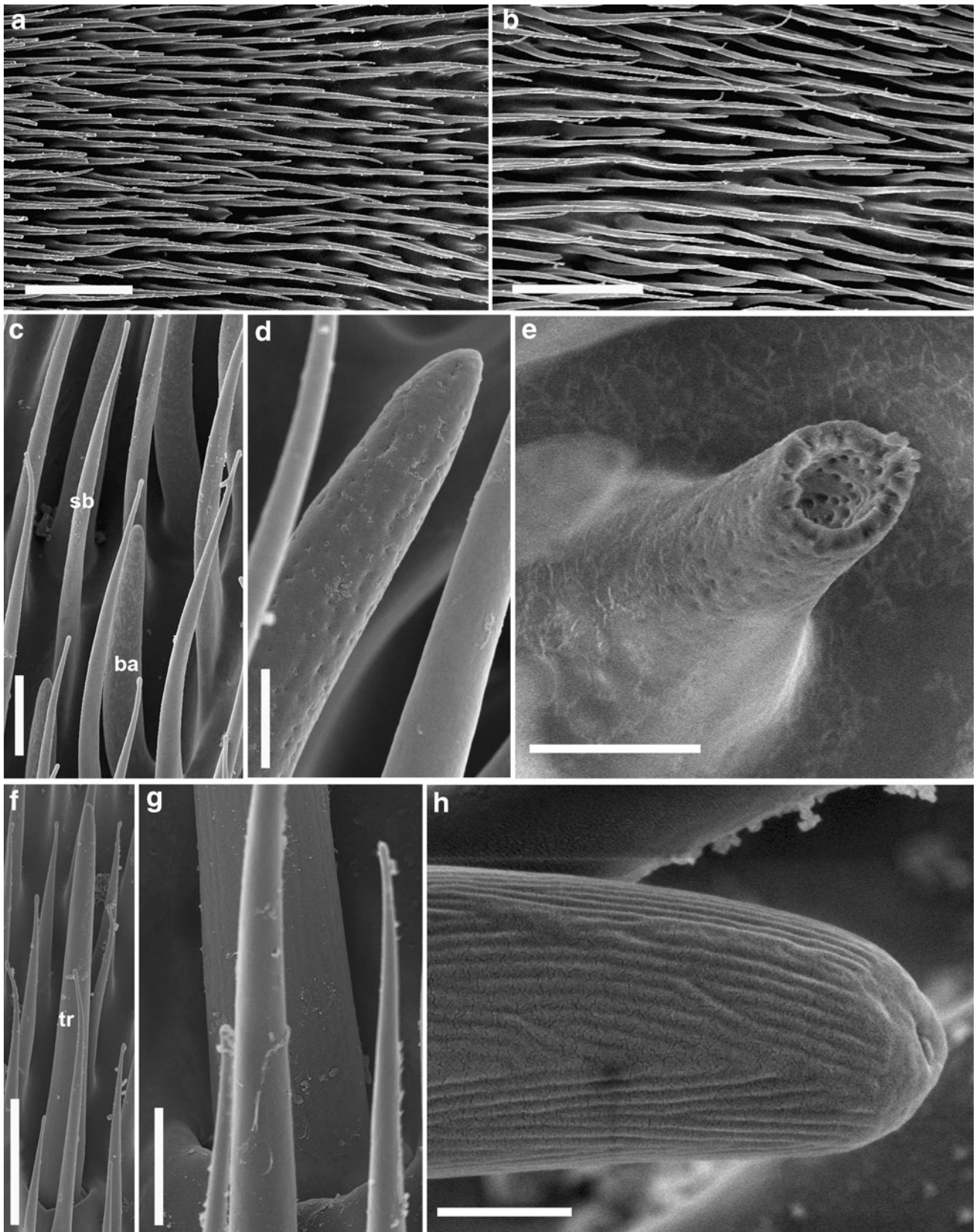
◀ **Fig. 3** SEM micrograph of postpedicel of *M. ruficauda*. **a** Cuticular surface of proximal zone (*scale bar* = 20 μm). **b** Cuticular surface of medial zone (*scale bar* = 20 μm). **c** Higher magnification of cuticular surface of postpedicel showing small bristles (sb) and basiconic sensilla (ba) (*scale bar* = 4 μm). **d** Basiconic sensillum (*scale bar* = 2 μm). **e** Cross-section of basiconic sensillum (*scale bar* = 2 μm). **f** Trichoid sensillum (tr) (*scale bar* = 10 μm). **g** Flexible insertion of trichoid sensillum (*scale bar* = 3 μm). **h** Tip of trichoid sensillum (*scale bar* = 500 nm)

Results

Morphology of the antennae

Mallophora ruficauda antennae have four segments: scape (I), pedicel (II), postpedicel (III) and a terminal stylus (IV), from proximal to distal end, resembling the general antennal morphology of other Asilidae species (Wood 1981) (Fig. 1).

The scape is a glabrous segment that exhibits two groups of ventral and dorsal "long bristles" with a socket at their base and deep longitudinal grooves with striated surface (Fig. 1b). Comparison between sexes did not reveal any statistical differences in this segment (Table 1). The pedicel showed groups of long bristles too, but in contrast to the scape, it has a pilose cover with a homogenous distribution of small bristles or microtrichias (Fig. 1b). Registered measurements showed that females have a wider pedicel than males, but there is no difference in their maximum length (Table 1). The postpedicel is longer than the pedicel and scape (Fig. 1b; Table 1). Also, the postpedicel maximum width is higher for females than males (Table 1). The terminal stylus is a rigid segment that lacks of any type of the structures mentioned above, being longer in females than in males (Fig. 1b; Table 1). Both females and males display stylate antennae without apparent morphological dimorphism. No differences between sexes were found when analysing the total length and width of the antennae (Table 1). Along the antennae surface, long bristles, small bristles, multiporous basiconic and uniporous trichoid sensilla, and various pits were observed (Figs. 1, 2).

Description of cuticular structures

Postpedicel shows four types of cuticular structures oriented towards the tip of the segment: small bristles, multiporous basiconic and uniporous trichoid sensilla, and sensory pits (Figs. 2, 3).

The entire surface of the postpedicel is densely covered with small bristles, which are hair-like structures that taper from the base to a bulbous tip (Fig. 3c). These are aporous structures with a fixed insertion, and occasionally, they present branches along their axis. Small bristles are distributed along the proximal, medial and distal zones of

Table 2 Measurements of postpedicel cuticular structures of the antenna of *M. ruficauda*

Sexes		♀ [N = 7]			♂ [N = 7]		
Postpedicel zones		Proximal	Medial	Distal	Proximal	Medial	Distal
Small bristles	Length	19.8–24.9 (22.2 ± 1.8) Aa	24.6–30.6 (27.3 ± 2.5) Ab	23.6–31.0 (26.1 ± 2.5) Ab	17.6–24.3 (20.3 ± 2.1) Aa	22.0–28.8 (25.5 ± 2.2) Ab	21.8–29.3 (24.8 ± 2.4) Ab
	Density	0.0254–0.0464 (0.0347 ± 0.0078) Aa	0.0196–0.0260 (0.2249 ± 0.0021) Ab	0.0177–0.0229 (0.0202 ± 0.0016) Ac	0.0307–0.0467 (0.0413 ± 0.0054) Ba	0.0208–0.0310 (0.0248 ± 0.0035) Bb	0.0198–0.0256 (0.0222 ± 0.0022) Bc
Basiconic sensilla	Length	–	13.7–17.4 (15.2 ± 1.4) Xx	14.0–17.9 (15.6 ± 1.5) Xx	–	13.8–16.0 (14.7 ± 0.8) Xx	13.9–16.1 (14.9 ± 0.8) Xx
	Density	–	0.0066–0.0098 (0.0084 ± 0.0010) Xx	0.0055–0.0092 (0.0078 ± 0.0012) Xx	–	0.0069–0.0140 (0.0104 ± 0.0023) Xx	0.0054–0.0125 (0.0085 ± 0.0025) Xx

The measurements ranges are followed between brackets by the mean and SD. Length measurements are in μm. Density is expressed as number of cuticular structures per μm². Measurements ranges followed by the same letter are not significantly different. Different letters indicate significant differences: A–B between sexes (repeated measure ANOVA, $p < 0.05$), a–c between zones (repeated measure ANOVA Pairwise Comparison, $p < 0.05$), X–Y between sexes (t test Independent Samples, $p < 0.05$), and x–y between zones (t test paired samples, $p < 0.05$). Between square brackets, the total number of measured individuals (*N*) is expressed

Table 3 Measurements of postpedicel pits of the antenna of *M. ruficauda*

PITS		♀[$N = 6$]	♂[$N = 6$]	$t; p$
SACK	Number	11.0–17.5 (13.5 ± 2.5)	9.50–17.00 (13.00 ± 3.08)	−0.49; 0.637
	Maximum length	24.57–41.06 (33.51 ± 6.76)	27.23–39.00 (31.21 ± 4.40)	−0.52; 0.617
	Maximum width	21.63–33.82 (27.87 ± 5.23)	19.74–27.83 (24.56 ± 2.88)	−1.14; 0.282
OVAL	Number	2.00–4.00 (2.83 ± 0.82)	2.00–5.00 (2.92 ± 1.16)	−0.04; 0.97
	Maximum length	10.64–15.62 (13.19 ± 1.82)	12.76–15.83 (14.15 ± 1.10)	1.21; 0.25
	Maximum width	9.46–17.14 (11.60 ± 2.92)	10.37–14.10 (12.13 ± 1.21)	0.85; 0.41
CLOUD	Number	9.00–12.00 (10.17 ± 1.12)	7.50–11.50 (9.67 ± 1.47)	−0.77; 0.46
	Maximum width	21.69–28.54 (25.35 ± 2.52)	23.42–35.65 (27.39 ± 4.38)	0.96; 0.36

The ranges of measurements are followed between brackets by the mean and SD. Length and width measurements are expressed in μm. Between square brackets, the total number of measured individuals (*N*) is expressed

postpedicel, and are longer in medial and distal zones both in females and males (Table 2). Regarding density, it decreases in small bristles from the proximal to the distal zone in both sexes, but are a little denser in males (Table 2).

The long bristles are packed in the dorsal and ventral margin of the scape and pedicel (Fig. 1b). In the scape, they are in groups of 8–17 in females (13.5 ± 1.8, $N = 6$) and of 9–19 in males (13.1 ± 3.0, $N = 5$). In females, these structures have a length of 0.18–0.22 mm (0.20 ± 0.01 mm, $N = 6$), while in males it is of 0.15–0.22 mm (0.19 ± 0.03 mm, $N = 5$). Regarding the pedicel, long bristles are found in groups in both dorsal and ventral side. In females, the long bristles are grouped from 3 to 6 units (4.9 ± 0.4, $N = 6$) with a length of 0.21–0.25 mm (0.23 ± 0.01 mm, $N = 6$). In the males, the long bristles are grouped from 3 to 7 units (4.4 ± 0.29, $N = 5$) with a length of 0.16–0.28 mm (0.21 ± 0.05 mm, $N = 5$). In order to identify whether number and/or length of long bristles can be considered as a sexually dimorphic character, we compared both measures between sexes with an independent *t* test. We only found differences between sexes in the number of long bristles in the pedicel ($t_{(0.025,9)} = 2.38, N = 11, p = 0.04$). On the contrary, we found no differences in the number of long bristles in the scape and their length in the scape and pedicel (number of long bristles in the scape: $t_{(0.025,9)} = 0.28, N = 11, p = 0.79$; length of the scape: $t_{(0.025,9)} = 0.62, N = 11, p = 0.55$; length of the pedicel: $t_{(0.025,9)} = 1.05, N = 11, p = 0.34$).

Basiconic sensilla are regularly scattered among small bristles and are peg-shaped structures with its shaft tapering abruptly in the tip. Each sensillum has a fixed insertion and uniformly distributed pores along the cuticular wall (Fig. 3c, d, e). These sensilla are present only in the medial and distal zones of postpedicel (Table 2, Fig. 3b), and we found that females have 3,700 and males 4,300 total basiconic sensilla. The average density of these sensilla in females in the medial zone is 0.0084 and 0.0078 per μm²

for the distal zone, whereas for males, it is of 0.0104 and 0.0085, respectively (Table 2). The average density in the postpedicel is of 0.0081 per μm² for females and 0.0094 for males. There are no differences in basiconic sensilla length or density among zones in each sex, neither for length nor for density between sexes (Table 2).

Trichoid sensilla are located in the proximal zone grouped in the latero-ventral region of the postpedicel, in groups of 6–8 sensilla in both sexes ($t_{0.025,7} = -0.59, N = 9, p = 0.57$) (Fig. 2e). These sensilla have a pore in the tip, a striated shaft and a flexible insertion, being their length 43.74 ± 2.81 μm ($N = 4$) for females and 40.67 ± 3.48 μm ($N = 5$) for males ($t_{0.025,7} = -0.04, N = 9, p = 0.97$) (Fig. 3f, g, h).

Sensory pits were observed in the proximal zone of the postpedicel in both sexes (Fig. 2a, b, c). By OM, we found three different types of morphological sensory pits: sack, cloud and oval (Fig. 2b, c), which are arranged mainly on the internal side of the antennae (Fig. 1b). There are no differences in number, length and width between sexes (Table 3). By SEM observations, we could not differentiate among morphological type of the pits, but we found multiporous basiconic sensilla inside them (Fig. 2d).

Discussion

In this work, we study the morphology of the antenna in adults of *M. ruficauda* emphasizing on the postpedicel olfactory sensilla. Our results show that adults of *M. ruficauda* have compressed antennae with four segments, and these results are in accordance with the few studies made on antennal morphology in Asilidae family (Wood 1981; Yeates 2002). Regarding the cuticular structures, there are long and small bristles type, multiporous basiconic and uniporous trichoid sensilla, and sensory pits, which were described in this study. Although there are no previous reports of antennal structures on the Asilidae family, these

Table 4 Presence and possible functions of cuticular structures found in *M. ruficauda* reported in other species of Brachycera

Cuticular structure / Function / Species	Long bristles Mechanosensorial spatial orientation	Small bristles Improving olfaction process by slowering and trapping air flow	Basiconic sensilla Chemosensorial detection of volatile cues	Sensory pits Chemosensorial detection of volatile cues	Reference
Anastrepha fraterculus (Wiedemann)		*	*		Bisotto-de-Oliveira et al. (2011)
Anastrepha serpentina (Wiedemann)	*	*		*	Castrejón-Gómez and Rojas (2009)
Ceratitis capitata (Wiedemann)		*	*		Mayo et al. (1987)
Chrysomya megacephala (Fabricius)	*	*	*	*	Sukontason et al. (2004)
Chrysomya nigripes Aubertin	*	*	*	*	Sukontason et al. (2004)
Chrysomya pinguis (Walker)	*	*			Sukontason et al. (2008)
Chrysomya rufifacies (Macquart)	*	*	*	*	Sukontason et al. (2004)
Cochliomyia hominivorax (Coquerel)	*	*	*		De Freitas Fernandes et al. (2004)
Delia radicum L.	*	*		*	Ross and Anderson (1987)
Dermatobia hominis (Linnaeus Jr.)	*	*			De Freitas Fernandes et al. (2002)
Drosophila melanogaster Meigen		*	*	*	Itoh et al. (1991); Stocker (2001)
Eupeodes volucris Osten Sacken		*			Hood Henderson and Wellington (1982)
Exorista sp. Meigen (parasitoid)	*		*		Abouzied (2008)
Gasterophilus nigricornis (Loew)		*	*	*	Zhang et al. (2012)
Hydrotaea chalcogaster (Wiedemann)	*	*	*	*	Sukontason et al. (2007)
Hydrotaea irritans (Fallen)	*	*	*	*	Been et al. (1988)
Hypoderma bovis De Geer	*	*	*	*	Hunter and Adserballe (1996)
Liriomyza sativae Blanchard	*	*		*	Zhao and Kang (2002)
Lucilia cuprina (Wiedemann)	*	*	*	*	Sukontason et al. (2004)
Metasyrphus venablesi (Curran)		*			Hood Henderson and Wellington (1982)
Musca autumnalis De Greer		*	*	*	Bay and Pitts (1976)
Musca domestica L.	*	*	*	*	Sukontason et al. (2004)
Neobellieria bullata (Parker)				*	Wasserman and Itagaki (2003)
Oestrus ovis L.		*		*	Poddighe et al. (2010)
Parasarcophaga dux (Thomson)	*	*	*	*	Sukontason et al. (2004)
Protophormia terraenovae Robineau-Desvoidy		*	*	*	Setzu et al. (2011)
Pseudacteon tricuspis Borgmeier (parasitoid)	*	*	*		Chen and Fadamiro (2008)
Pseudoperichaeta nigrolineata Walker (parasitoid)	*	*	*		Rahal et al. (1996)
Sarcophaga argyrostoma Robineau-Desvoidy		*		*	Slifer and Sekhon (1964)
Trichopoda pennipes Fabricius (parasitoid)	*	*	*		Giangiuliani et al. (1994)

Notation: *, presence

structures resemble those found in several species of Brachycera, allowing us to believe in a bauplan regarding olfaction in higher Diptera species (Table 4).

Mallophora ruficauda presents long bristles that are present also in several species of Diptera (Table 4). These structures comprise a mechanosensory function and could be involved in antennae spatial orientation, suggesting that could be important during the oviposition in *M. ruficauda*, since females touch the supports with their antenna and this could determine oviposition microsite selection (M.K. Castelo, personal observation). Small bristles in *M. ruficauda* pedicel and postpedicel show a morphology and a structure similar to microtrichia, non-innervated hair-like projections that cover antennal segments of several species of Brachycera (Table 4). No sensory function had been assigned to small bristles, but some authors have suggested the possibility that microtrichia found in the postpedicel could modify the air flow. Biomechanical approach asserts that dense arrays hair-like projections decrease flow velocity allowing odorants molecules diffuse onto sensorial hair for a longer time, increasing capture by them (Hood Henderson and Wellington 1982; Cheer and Koehl 1987a, b; Ross and Anderson 1987; Loudon 1995; Koehl 1996; Schneider et al. 1998; Loudon and Koehl 2000; Loudon 2003; Loudon and Davis 2005). Moreover, since small bristles cannot interact with chemical stimuli that arrive at the antenna, these structures could enhance chemoreceptors interception of odorant molecules by decreasing interference between these sensorial hairs (Loudon 2003). Thus, small bristles covering the postpedicel could enhance the efficiency of olfaction in brachyceran species since, in contrast to other insects, this antennal segment is the only one bearing olfactory sensilla.

Both sensilla types found in *M. ruficauda* postpedicel, multiporous basiconic and uniporous trichoid are characterized by the presence of pores, and by this characteristic, they are considered chemoreceptors: multiporous basiconic sensilla are associated with olfaction; meanwhile, the uniporous trichoid sensilla are related to gustatory-tactile function (Slifer 1970; Zacharuk 1980; Ryan 2002). As described in previous reports for other insects, both chemosensilla are generally located on the postpedicel. The multiporous basiconic sensilla found in *M. ruficauda* are similar to those present in other dipteran species (Table 4). This sensillum is associated with location of nutritional resources and oviposition site in several species of Diptera. Particularly in parasitoids flies, some authors linked them to the detection of chemical cues from host (Been et al. 1988; Giangiuliani et al. 1994; Stocker 1994; Rahal et al. 1996; De Freitas Fernandes et al. 2004; Chen and Fadamiro 2008). In *M. ruficauda,* these sensilla are distributed in both lateral sides of the postpedicel and would be the unique type of sensillum involved in detection of olfactory cues. In contrast, most dipteran species studied show several subtypes of olfactory sensilla (Bay and Pitts 1976; Mayo et al. 1987; Been et al. 1988; Hunter and Adserballe 1996; Rahal et al. 1996; De Freitas Fernandes et al. 2004; Sukontason et al. 2004, 2007; Chen and Fadamiro 2008; Setzu et al. 2011; Zahng et al. 2012). Based on our findings, no evidence of different sensillum subtypes was found, which suggests that for this fly species, there are few substances with biological relevance to detect, maybe just the host cues.

Regarding uniporous trichoid sensilla composed by a simple porous and a flexible insertion related to mechanosensitive function (Slifer 1970; Zacharuk 1980; Ryan 2002). There are some studies showing that these sensilla can detect volatiles (Slifer 1970). However, it has not been previously described a similar structure in any antennal segment of any other brachyceran species being ours the first report of this type of bimodal sensilla on their antennae. On the other hand, many species of hymenopteran parasitoids show this type of sensilla, which seems to be essential in host discrimination and acceptance. Particularly, female wasps that parasitize hidden hosts detect host's chemical contact cues using these type of sensilla during the typical drumming behaviour of their antennae (Pettersson et al. 2001; Benedet et al. 2002; Roux et al. 2005; Gao et al. 2007; Van Baaren et al. 2007; Da Rocha et al. 2007; Onagbola and Fadamiro 2008). Therefore, the presence of this type of structure might be related to the oviposition strategy of *M. ruficauda*, where female might be detecting quality cues of egg cluster's supports or other females's cues placing their antenna over oviposition substrates (M.K. Castelo, personal observation). In this way, females could be selecting oviposition sites based on other female decisions, since in the field it is common to see egg clusters one above another and grouped on the same vegetation supports.

When comparing antennae of other dipteran parasitoids with the antennae of *M. ruficauda,* we found resemblances and dissimilarities. As in the male of Tachinidae parasitoid *Exorista* sp., only basiconic sensilla were observed in the postpedicel (Abouzied 2008). No other dipteran parasitoids present a unique type of olfactory sensillum in the postpedicel, which generally show a minimum of 3 types (Giangiuliani et al. 1994; Rahal et al. 1996; Chen and Fadamiro 2008). In these parasitoids, olfactory cues are relevant in nutritional resources location, in contrast to *M. ruficauda* who uses mainly visual cues in order to hunt their prey (Peterson 1981; Wood 1981; Musso 1983; Stireman III et al. 2006). Therefore, the several types of olfactory sensilla may help in the detection and recognition of complex chemical cues related to the search of resources. Despite this, *M. ruficauda* antenna is similar to ones described in other dipteran parasitoids in total number of

sensilla and in the presence of shallow depressions or pits (Giangiuliani et al. 1994; Rahal et al. 1996; Chen and Fadamiro 2008). In *M. ruficauda,* these pits have different forms (i.e. single- or multichambered) and contain olfactory sensilla, as it was found in the parasitoid *Trichopoda pennipes* Berthold (Tachinidae; Giangiuliani et al. 1994). However, pits presence in the postpedicel is not a characteristic restricted to dipteran parasitoids. In several higher Diptera, these structures are considered to be involved in olfaction based on morphology, presence of olfactory sensilla and studies on their physiological behaviour (Table 4). Moreover, in some species, the number and morphology of sensory pits are considered as sexual dimorphic characters (Slifer and Sekhon 1964; Bay and Pitts 1976; Rahal et al. 1996; Sukontason et al. 2004); however, this was not observed in *M. ruficauda.*

Sexual dimorphism in antennae of Diptera is moderated and probably related to different functions and/or roles of females and males (Slifer and Sekhon 1964; Bay and Pitts 1976; Mayo et al. 1987; Ross and Anderson 1987; Stocker 1994, 2001; Rahal et al. 1996; De Freitas Fernandes et al. 2002, 2004; Sukontason et al. 2004; Chen and Fadamiro 2008; Setzu et al. 2011). For instance, location of oviposition sites in females or a mating partner in males (Rahal et al. 1996; De Freitas Fernandes et al. 2002, 2004). In *M. ruficauda,* we found more basiconic sensilla in males than in females, and this could be related to a higher sensitivity in the detection of pheromones involved in the reproductive behaviour (Chapman 1982). Regarding the maximum width of the pedicel, postpedicel and maximum length of the flagellum, they were higher in females than in males. However, since there exists also a difference in the insect body size, these may not have a biological relevance. Finally, we found along the three zones sampled a higher density of small bristles in males than in females. These differences in the antennal microstructure may have an effect in olfaction.

In conclusion, we characterized the chemoreceptors found in the antennae of *M. ruficauda.* We found two types of structures related to the detection of volatile compounds: multiporous basiconic sensilla and sensory pits. Given the high amount of basiconic sensilla and sensory pits, the detection of host chemical cues from a long distance could be achieved with these structures. Also, the sensitivity of these structures could be enhanced by the presence of small bristles that slow down the air flow going through the antenna, thus reducing the interference between the sensilla and concentrating the odour molecules reaching the pits. Once female arrives at the host habitat where hosts are distributed in patches in the soil (i.e. host microhabitat), and after selecting the oviposition height, the choice of an oviposition site on the substrate could be mediated by mechanoreceptors located in the antenna, because females oviposit on the underside of the substrates, with the latero-ventral zone of the postpedicel of both antennae touching the surfaces. Additionally, only one type of basiconic sensillum was found in both sexes suggesting that they detect the same kind of odours, as found in other species of Diptera (Hallberg et al. 1984; Zhao and Kang 2002). Although chemical cues detected were the same, females might locate hosts by these cues, while males might be searching females by detecting hosts. Hence, females might be using signals in an oviposition context while males would do it under a mating context. Moreover, a higher number of sensilla and density of small bristles were found in males than in females suggesting that males are more sensitive to odours than females. Finally, regarding trichoid sensilla, they were found on both sexes and they could be involved in mating behaviour. Males could use these sensilla in the recognition of a female, and they could be involved in courtship because it has been seen that males tap the female with its antennae during it. Still, further studies are needed to determine which type of volatiles adults detect and whether they are similar for both sexes. However, the large number of olfactory structures on the antennae described in the present study supports the hypothesis of paramount importance of olfactory cues in life history of *M. ruficauda.*

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